

Expression of Follistatin-Related Genes Is Altered in Heart Failure

Enrique Lara-Pezzi, Leanne E. Felkin, Emma J. Birks, Padmini Sarathchandra, Kalyani D. Panse, Robert George, Jennifer L. Hall, Magdi H. Yacoub, Nadia Rosenthal, and Paul J. R. Barton

Harefield Heart Science Centre (E.L.-P., L.E.F., E.J.B., P.S., K.D.P., R.G., M.H.Y., N.R., P.J.R.B.), National Heart and Lung Institute, Imperial College London, and Harefield Hospital (E.J.B., R.G.), Royal Brompton and Harefield National Health Service Trust, London UB9 6JH, United Kingdom; Mouse Biology Unit (E.L.-P., N.R.), European Molecular Biology Laboratory, 00015 Monterotondo, Italy; and Cardiovascular Division (J.L.H.), Department of Medicine, University of Minnesota, Minneapolis, Minnesota 55455

Follistatins play roles in diverse biological processes including cell proliferation, wound healing, inflammation, and skeletal muscle growth, yet their role in the heart is currently unknown. We have investigated the myocardial expression profile and cellular distribution of follistatin (FST) and the FST-like genes *FSTL1* and *FSTL3* in the normal and failing heart. Expression was further analyzed in the novel setting of recovery from heart failure in myocardium obtained from patients who received combined mechanical (left ventricular assist device) and pharmacological therapy. Real-time PCR revealed that *FSTL1* and *FSTL3* expression was elevated in heart failure but returned to normal after recovery. *FSTL3* expression levels correlated with molecular markers of disease severity and *FSTL1* with the endothelial cell marker CD81, suggesting a potential link with vascularization. *FSTL1* levels before treatment correlated with cardiac function after recovery,

suggesting initial levels may influence long-term outcome. Immunohistochemistry revealed that FST was primarily localized to fibroblasts and vascular endothelium within the heart, whereas *FSTL1* was localized to myocytes, endothelium, and smooth muscle cells and *FSTL3* to myocytes and endothelium. Microarray analysis revealed that FST and *FSTL1* were associated with extracellular matrix-related and calcium-binding proteins, whereas *FSTL3* was associated mainly with cell signaling and transcription. These data show for the first time that elevated myocardial expression of FST-like genes is a feature of heart failure and may be linked to both disease severity and mechanisms underlying recovery, revealing new insight into the pathogenesis of heart failure and offering novel therapeutic targets. (*Endocrinology* 149: 5822-5827, 2008)

HEART FAILURE REMAINS a major cause of morbidity and mortality and is characterized by molecular, cellular, and physiological changes in the myocardium resulting in cardiac myocyte hypertrophy, chamber dilation, and adverse ventricular remodeling leading ultimately to end-stage dilated cardiomyopathy (1). Although generally considered unidirectional, it is now recognized that myocardial remodeling and end-stage heart failure can in some cases be reversed after mechanical unloading of the heart using left ventricular assist devices (LVAD). We recently described a combination therapy in which LVAD and pharmacological agents were used in an attempt to maximize recovery in end-stage dilated cardiomyopathy patients with two thirds of patients showing improvement sufficient to allow LVAD removal (2). The molecular mechanisms underlying heart failure and its reversal remain unclear.

Follistatin (FST) and the related proteins FST-like-1 (*FSTL1*), also known as TSC-36/FRP/Fik) and *FSTL3* (also known as FRP/FLRG) act by neutralizing activins, members of the TGF β family that are implicated in diverse biological processes including cell proliferation and differentiation, wound healing,

inflammation, and fibrosis (3). Activins may contribute directly to the pathogenesis of heart failure because they are elevated in serum from heart failure patients, are increased in cardiomyocytes after experimental myocardial infarction, and can act to inhibit sarcomere organization (4, 5). In addition to their role in regulating activin, follistatins have been implicated in skeletal muscle regeneration (6) and are antagonists of myostatin, another TGF β family member that plays a key role in regulating skeletal muscle growth. Although originally thought to be skeletal muscle specific, myostatin has recently been shown to be up-regulated in the heart after myocardial infarction (7), to block cardiomyocyte growth *in vitro* (8), and to have a developmental profile consistent with regulation of perinatal cardiac muscle growth (9). In contrast, the expression and role of follistatins in the adult heart has remained unexplored, although recent reports have described expression during early heart development in chick and mouse (10-12). We hypothesized that follistatins may be altered in heart failure and thereby contribute to altered signaling. Here, we examine the expression of FST, *FSTL1*, and *FSTL3* in normal and failing heart and after recovery from heart failure using a combination of real-time PCR, microarray, and immunocytochemical analysis.

First Published Online July 10, 2008

Abbreviations: FST, Follistatin; *FSTL1*, FST-like-1; GO, gene ontology; LVAD, left ventricular assist device; SPARC, secreted protein acidic, rich in cysteine.

Endocrinology is published monthly by The Endocrine Society (<http://www.endo-society.org>), the foremost professional society serving the endocrine community.

Materials and Methods

Myocardial samples

Samples were obtained after informed consent and with the approval of the Royal Brompton and Harefield Research Ethics committee, and

the study conforms with the principles outlined in the Declaration of Helsinki. All patients had dilated cardiomyopathy without histological evidence of myocarditis. Left ventricular myopathy was collected at the time of surgical LVAD implantation (implant, $n = 27$) in patients with end-stage heart failure. These samples therefore represent a cohort of end-stage heart failure samples. Biopsies were also obtained from donor hearts with good hemodynamic function and used for transplantation. These samples therefore represent normal myocardium (donor, $n = 9$). For the specific analysis of myocardial recovery, samples were obtained from patients at the time of implantation of the LVAD device (implant, $n = 13$). After recovery from heart failure, samples were taken at the time of LVAD removal (explant, $n = 13$) and again 1 yr after explant (explant + 1 yr, $n = 9$). Similarly, for patients who failed to recover after LVAD combination therapy, samples were taken at LVAD implantation and LVAD explantation of the device (implant and explant, respectively, $n = 5$) (2).

Real-time PCR

Total RNA was extracted from frozen samples using RNeasy mini columns (QIAGEN, Crawley, UK), deoxyribonuclease-treated, and used in reverse transcription reaction with random priming (Applied Biosystems, Warrington, UK) as described (13). Real-time PCR was performed using the following off-the-shelf TaqMan assays (Applied Biosystems): human FST (Hs00246260.m1), FSTL1 (Hs0020063.m1), FSTL3 (Hs0061065.m1), INHBA (Hs00170103.m1), INHA (Hs00171410.m1), CD31 (Hs0165276.m1), and ACTA1 (Hs00599403.m1) and rat FST (Rn01561225.m1), Fstl1 (Rn01474870.m1), and Fstl3 (Rn00866203.m1).

The primers used for the BNP PCR were 5'-gaggaagatggaacacgacga-3' and 5'-tggttggaacacagacagagctgct-3', and the FAM-labeled probe was 5'-cagcagcttgacagacacga-3'. Data were analyzed using the comparative cycle threshold method normalizing to 18S rRNA and presented as mean \pm sd. Paired or unpaired *t* tests assuming unequal variance were used to test for significant changes between the patient groups, and Spearman's rank test was used to identify correlations between genes.

Immunohistochemistry

Myocardium from transplant donors and heart failure patients was embedded in OCT and frozen in liquid nitrogen. Frozen sections (5 μ m) were fixed in acetone, washed in PBS, and blocked with 0.3% H₂O₂ in PBS for 10 min followed by 3% BSA in PBS for 30 min. Sections were incubated separately for 1 h with antibodies against FST, FSTL1, and FSTL3 (all at 1:50 dilution; R&D Systems, Minneapolis, MN) followed by biotinylated goat antimouse Ig for FST and FSTL1 or biotinylated rabbit anti-goat diluted for FSTL3 (1:250; Vector Laboratories, Burlingame, CA) and avidin-biotin complex (Vector). Reactivity was detected using diaminobenzidine tetrahydrochloride (Sigma Chemical Co., St. Louis, MO; 25 mg/ml) and hydrogen peroxide (0.01% wt/vol). Sections were then counterstained with hematoxylin. The specificity of the antibodies was tested by Western blot analysis (supplemental Fig. 2, published as supplemental data on The Endocrine Society's Journals Online web site at <http://endo.endojournals.org>).

Microarray analysis

RNA ($n = 16$) was isolated from paired implant and explant samples from six recovery and two nonrecovery patients. cRNA synthesis and array hybridization were performed as previously described on the Affymetrix HG-U133A gene chip (14) and data analyzed using the GeneSnp 7.2 suite (Agilent, Santa Clara, CA). For the generation of gene expression correlation lists, all 16 samples were normalized against the eight implant samples together and then interpreted as individual samples. The expression profile of each FST gene was compared with that of every other gene in the array, and lists of 100–150 genes showing correlation with the lowest *P* values were generated. These correlation lists were compared with other correlation lists and to preexisting gene ontology (GO) lists, distributed into three categories: biological process, molecular function, and cellular compartment. Those with a similarity *P* value < 0.005 and more than three genes in common with the FST list were selected. The *P* value represents the probability that genes appearing in both lists at the same time do so by chance. For each selected GO list in each category, the number of genes in common with the FST

list was represented as the percentage of genes compared with the total number of common genes in all the lists represented.

Experimental pressure overload

Pressure overload hypertrophy was induced by thoracic aortic banding as previously described (15) with an increase in the left ventricular mass index of $49.7 \pm 5.1\%$ compared with sham-operated animals. Total RNA was prepared from left ventricular free wall and analyzed by real-time PCR as above.

Results

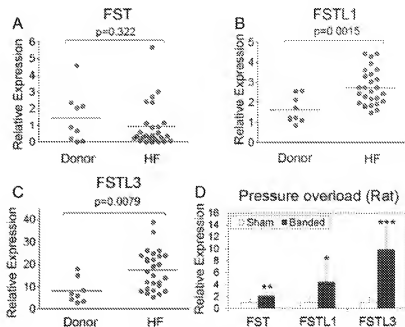
We examined the expression levels of FST, FSTL1, and FSTL3 in myocardial samples of end-stage heart failure and from healthy donors and found that both FSTL1 and FSTL3 were elevated in heart failure, whereas FST expression was unchanged (Fig. 1). Analysis of actin (A subunit, INHBA) and inhibin (INHA) expression showed no difference between end-stage heart failure patients and healthy donors (supplemental Fig. 1). In addition, the expression of myostatin was examined and found to be below reliably detectable levels by quantitative PCR (data not shown). To confirm that up-regulation of FSTL1 and FSTL3 is a general feature of heart failure, we further analyzed the expression of the three FST molecules in a rat model of pressure overload-induced hypertrophy. As shown in Fig. 1D, both FSTL1 and FSTL3, and to a lesser extent FST, were strongly induced 3 wk after thoracic aortic banding, compared with sham-operated rats.

To determine their cellular distribution, sections of myocardium from both donors and heart failure patients were stained with antibodies specific for FST, FSTL1, and FSTL3 (Fig. 2). FSTL1 and FSTL3 staining was clearly seen in myocytes, the major cell type present in myocardium. Consistent with the gene expression data, staining of FSTL1 and FSTL3 proteins was significantly more intense in heart failure samples compared with donors. Both FSTL1 and FSTL3 were also evident in vascular endothelial cells of capillaries and small vessels within the myocardium. FSTL1 was also present in smooth muscle cells of larger vessels (Fig. 2F). Neither FSTL1 nor FSTL3 were present in interstitial cells (fibroblasts) in the sections examined. In contrast, FST expression was evident in both perivascular interstitial cells (fibroblasts) and vascular endothelial cells but was not evident in cardiac myocytes.

Because their expression was elevated, we looked for correlation between the expression of FSTL1 and FSTL3 and expression of other molecular markers within the heart failure group. FSTL3 correlated positively with both skeletal α -actin (ACTA1) and brain natriuretic protein (BNP), both markers of heart failure disease severity (Fig. 3). In contrast, FSTL1 levels showed a negative correlation with skeletal α -actin, suggesting contrasting roles for FSTL1 and FSTL3 in the failing heart. FSTL1 levels also showed positive correlation with the endothelial cell marker CD31, suggesting a potential link with extent of vascularization.

Given the elevation of FSTL1 and FSTL3 in heart failure patients, we further examined expression levels in the special case of myocardial recovery from heart failure in a group of patients who received a combination of mechanical unloading (LVAD) and pharmacological therapy (2). Here, paired myocardial samples were available from failing hearts taken

Fig. 1. FSTL1 and FSTL3 expression is induced in heart failure. FST (A), FSTL1 (B), and FSTL3 (C) expression was determined using real-time RT-PCR in myocardial samples from donor hearts (Donor, $n = 9$) and heart failure patients (HF, $n = 27$). Results are expressed in relative units, and the P value (nonparametric unpaired t test) is given for each comparison. D, Fst, Fstl1, and Fstl3 expression was analyzed in rat heart 3 wk after thoracic aortic banding (black bars) or sham operation (white bars). *, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.0005$ (parametric unpaired t test, $n = 7$).



at the time of LVAD implantation (Implant) and resampled from the same heart after treatment (Explant and Explant + 1 yr). In patients who recovered, levels of both FSTL1 and FSTL3 returned to normal (Fig. 4, A–C). Samples were also available from five patients who failed to recover. Although the number of cases is small, we saw no significant change in FST, FSTL1, or FSTL3 during treatment (data not shown). However, it was noted that patients who did not recover had significantly higher level of expression of FSTL3 at the time of LVAD explant compared with recovery patients (15.44 ± 3.33 cf. 10.15 ± 5.2 , $P = 0.05$), further indicating the association of elevated FSTL3 with heart failure. Expression levels of FSTL1 and FSTL3 in recovery patients were also compared with available clinical data (2). From this it became apparent that higher FSTL1 levels at the time of LVAD implant cor-

related with significantly higher ejection fraction measured after treatment (Fig. 5), suggesting initial levels may have an influence on long-term outcome. FSTL3 showed an opposite trend, although the correlation was not significant.

To gain further insight into their potential physiological role in the heart, we analyzed microarray data on paired myocardial samples taken from patients at the time of LVAD implant and again at explant (14). We compiled lists of genes whose overall expression correlated with FST, FSTL1, or FSTL3 and compared these to preexisting GO lists (supplemental Table 1 and Fig. 3). Expression of both FST and FSTL1 correlated with genes encoding extracellular matrix constituents, adhesion molecules, genes expressed during embryonic development, and those encoding calcium-binding proteins. It is noteworthy that the lists of genes correlating with

Fig. 2. Cardiac immunohistochemical staining of FST, FSTL1, and FSTL3 in sections of normal and failing hearts. Myocardial tissue from donors (A–D) and heart failure patients (E–L) was fixed, sectioned, and incubated with antibodies specific for FST, FSTL1, or FSTL3 (or no primary antibody as a negative control), followed by biotin-conjugated secondary antibodies and horseradish peroxidase-avidin. Multiple fields of view were examined, and those including small myocardial vessels were chosen to illustrate the range of cellular staining (A–H, $\times 20$; I–L, $\times 40$). FST immunostaining was observed in fibroblasts, smooth muscle cells, and endothelium (E and I); FSTL1 in myocytes, endothelium; and smooth muscle cells (F and J); and FSTL3 in myocytes and endothelium (G and K). FSTL3 staining was also evident in nuclei of cardiac myocytes (arrowheads in G and K). C, Cardiac myocyte; E, endothelium; F, fibroblast; SM, smooth muscle cell. Bar, 100 μ m. The specificity of each antibody was verified by Western blot analysis (supplemental Fig. 2).

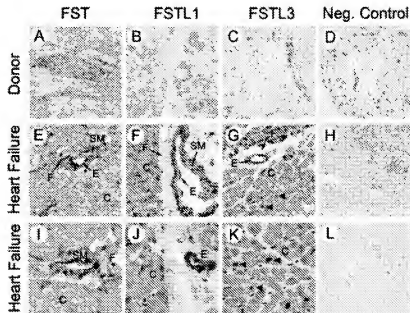
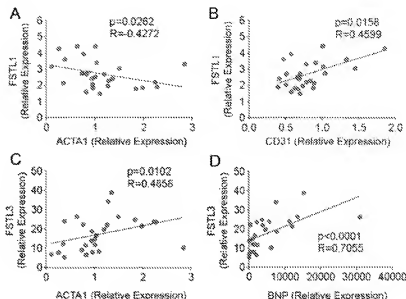


FIG. 3. Correlation between the expression of FSTL1 and FSTL3 with different markers in heart failure. Levels of FSTL1, FSTL3, ACTA1 (α -skeletal actin), CD31 (PECAM), and BNP expression in myocardial samples from heart failure patients were determined by real-time RT-PCR, and the Spearman correlation between the different markers was analyzed.



FST and FSTL1 are highly similar ($P < 10^{-54}$), suggesting they are involved in similar biological functions despite their contrasting cellular compartmentalization. In contrast, FSTL3 showed a distinct correlation profile associating particularly with genes encoding nuclear proteins and involved in cell signaling and transcription regulation (supplemental Fig. 3, G–I). It was also apparent that the list of FSTL3-correlating genes was similar to that of genes correlating with BNP ($P < 10^{-17}$) in agreement with the real-time PCR data across the whole of the recovery group. Consistent with the results of immunocytochemistry, the list of genes correlating with FST showed strong similarity to that correlating with the fibroblast marker Thy-1 ($P < 10^{-78}$), and the list of genes correlating with FSTL1 showed strong similarity to that correlating with α -smooth muscle actin (α -SMA; $P < 10^{-110}$).

Discussion

Follistatins have previously been implicated as regulators of the activin signaling pathway in muscle development, principally through their ability to inhibit myostatin, a pow-

erful negative regulator of growth. Follistatins have also been directly linked to tissue regeneration after injury in both skeletal muscle (6) and liver (16), making them interesting potential targets for myocardial repair. Despite this, the role of FST and FST-related genes in heart failure and their ability to influence repair and recovery of injured myocardium has to date been unexplored. The data reported here show that FSTL1 and FSTL3 are up-regulated in heart failure and return to normal after myocardial recovery. Their respective expression profiles and tissue distribution suggest contrasting roles in the heart. Both FSTL1 and FSTL3 were up-regulated in cardiac myocytes in the failing heart with expression also evident in endothelial cells and, in the case of FSTL1, in smooth muscle cells. In contrast, FST was not seen in myocytes but was localized to fibroblasts and endothelial cells and did not change in failure.

Follistatins may have beneficial effects in heart failure because they are antiinflammatory by virtue of their ability to down-regulate activin signaling. Activin is elevated in systemic inflammation (3), has been shown to be elevated in

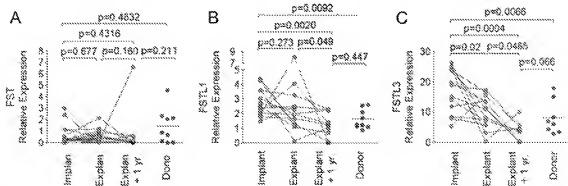


FIG. 4. FSTL1 and FSTL3 expression is normalized in recovery. FST (A), FSTL1 (B), and FSTL3 (C) expression was determined using quantitative real-time RT-PCR in myocardial samples from donor hearts ($n = 9$) and from heart failure recovery patients at the time of LVAD implant (Implant, $n = 13$), at the time of LVAD explant (Explant; $n = 13$), and again 1 yr after explant (Explant + 1 yr, $n = 9$). Results are expressed in relative units, and the P value is given for each comparison (paired t test for comparison of each patient at different time points; unpaired t test for comparison with the donor group). The difference between the means of the Explant + 1 yr group and the donor group was not significant both for FSTL1 and FSTL3.

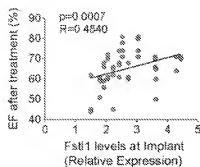


FIG. 5. The levels of FSTL1 at LVAD implant correlate with ejection fraction after recovery. FSTL1 levels in myocardial samples from heart failure patients ($n = 13$) taken at the time of LVAD implant were determined by real-time RT-PCR and correlated with their ejection fraction (EF, percent) measured using M-mode echocardiography before the explant (with the LVAD device off for 15 min before measurement; $n = 12$) and again at 1, 2, and 5 yr after LVAD explant ($n = 12$, $n = 13$, and $n = 9$, respectively), using Spearman's correlation test.

an ischemic model of heart failure in rats (4), and has negative effects on cardiac myocyte myofibrillogenesis (5). We did not detect a difference in myocardial actin expression between the end-stage (dilated cardiomyopathy) and donor samples analyzed here, suggesting that expression may be altered by ischemia rather than heart failure *per se*. Increased FST expression may act to reduce overall actin signaling in the myocardium. In this respect, it is also noteworthy that both FST and FSTL3 are themselves up-regulated by both actin and TGF β via SMAD activation in a negative feedback loop limiting actin signaling (17). Follistatins may also act through their ability to inhibit myostatin. For example, myostatin is known to inhibit development and growth in skeletal muscle, and inhibition by FST or loss of activity results in enhanced regeneration and reduced fibrosis after injury (18). Whether similar mechanisms function within the heart is currently unclear. Recent reports have suggested that myostatin is expressed during cardiac development (9) and that it may be up-regulated in heart failure (7, 8, 19), although its role in adult heart remains unclear (8, 19). In the samples analyzed here, myocardial levels of myostatin were very low or below detection, and no clear changes were evident in heart failure patients. Follistatins may also act by modulating BMP signaling. We examined our array data of paired samples at implant and explant and found no evidence of altered BMP gene expression at either high (intersect of paired and unpaired *t* test analysis, $P < 0.01$) or medium (paired *t* test, $P < 0.01$) stringency (14).

Their cellular distribution and expression profiles suggest contrasting roles within the myocardium. FST and FSTL1 appear to be associated with the extracellular matrix compartment and protein-binding functions. Although unchanged in heart failure overall, FST expression was associated with fibroblast markers and extracellular matrix proteins and regulators, suggesting that it may still play a role during the remodeling process. FSTL1 expression was associated with extracellular matrix genes. FSTL1 also shares structural similarity with secreted protein acidic, rich in cysteine (SPARC, also known as osteonectin/BM-40) (20), a matricellular protein involved in cardiac development and wound repair that regulates cell-extracellular matrix inter-

actions (21). In the samples analyzed here, the list for FSTL1 showed high similarity with those of matricellular proteins such as SPARC ($P = 3.91 \times 10^{-108}$) and hevin ($P = 3.68 \times 10^{-30}$), suggesting that they may be involved in similar functions. In this regard, matricellular proteins play a key role in wound healing and can have both positive and negative effects on angiogenesis (21). Interestingly, SPARC cleavage by metalloproteinases results in the release of peptides from its FST domain that stimulate angiogenesis (22). The notable association between FSTL1 and CD31 in the heart failure patients, together with the localization of FSTL1 in endothelial cells, suggests that it may have a similar role in angiogenesis. Both inflammatory and antiinflammatory roles have been proposed for FSTL1 (23, 24), but we found no evidence of significant correlation between the list of FSTL1-associated genes and markers of inflammation. This, together with the association with extracellular matrix-related genes suggests the principal role for FSTL1 in heart failure relates to extracellular matrix and cell adhesion regulation. An intriguing observation from the data presented here is the correlation of FSTL1 levels at implant with subsequent ejection fraction measured after recovery, suggesting a beneficial effect of initial FSTL1 on outcome. The mechanisms of such an effect remain unknown but may involve modulation of the response to the combination therapy protocol, such as a predisposition of the extracellular matrix to repair mechanisms. Although the mechanisms of such an effect remain unknown, recent data suggest that FSTL1 exerts a protective role on the myocardium, reducing the infarct size after ischemia-reperfusion injury in rodents (25). Of note, the antiapoptotic effect of FSTL1 is mediated by phosphatidylinositol 3-kinase and ERK signaling pathways, resembling the prosurvival effect of SPARC (26). Thus, up-regulation of FSTL1 in human heart failure may represent a compensation mechanism elicited by the heart to contain the damage.

FSTL3 expression in the adult is enriched in heart, lung, and kidney (3), and FSTL3 knockout mice have multiple abnormalities including hypertension and increased heart weight to body weight ratio (27). In the heart failure patients studied here, myocardial FSTL3 was elevated, and levels correlated with α -skeletal actin and BNP, both markers of disease severity. Unlike FST and FSTL1, the expression profile of FSTL3 on microarray shows a clear association with the nuclear compartment and with genes involved in signaling and transcription. This is consistent with previous studies that have noted that FSTL3 can be found in both the nucleus and cytoplasm of cell lines in addition to being secreted (28) and with the observation that FSTL3 nuclear labeling was detected in myocytes in the experiments presented here. Taken together, these observations raise an intriguing potential link between extracellular matrix signaling and cardiac myocyte gene regulation orchestrated by altered local FST expression.

In conclusion, our data show for the first time that elevated expression of the FST-related genes FSTL1 and FSTL3 is a feature of heart failure and that this is reversed on myocardial recovery after combination therapy. The expression profiles and cellular localization of FST, FSTL1, and FSTL3 argue for differing functions within the myocardium with FST and FSTL1 associated mainly with extracellular matrix

and protein binding, whereas FSTL3 was associated with the nucleus and gene regulation. The data point to new potential targets for therapeutic intervention in the treatment of heart failure.

Acknowledgments

Received February 1, 2008. Accepted June 30, 2008.

Address all correspondence and requests for reprints to: Dr. Paul J. R. Barton, Harefield Heart Science Centre, Hill End Road, Harefield, Middlesex UB9 6JH, United Kingdom. E-mail: p.barton@imperial.ac.uk.

This work was supported by research grants from the Magdi Yacoub Institute, Royal Brompton and Harefield Charitable Trustees, the European Molecular Biology Laboratory, the Spanish Centro Nacional de Investigaciones Cardiovasculares, and the Marie Curie Grant program.

Disclosure Summary: E.L.-P., L.E.F., E.J.B., P.S., K.D.P., R.G., J.L.H., M.H.Y., N.R., and P.J.B. have nothing to declare.

References

- Pfeffer MA, Braunwald E 1990 Ventricular remodeling after myocardial infarction. Experimental observations and clinical implications. *Circulation* 81: 1161–1172.
- Birks EJ, Tansley PD, Hardy J, George RS, Bowles CT, Burke M, Banner NR, Khaghani A, Yacoub MH 2006 Left ventricular assist device and drug therapy for the reversal of heart failure. *N Engl J Med* 355:1873–1884.
- Harrison CA, Gray PC, Vale WW, Robertson DM 2005 Antagonists of activin signaling: mechanisms and potential biological applications. *Trends Endocrinol Metab* 16:73–78.
- Yndestad A, Ueland T, Ole E, Florholmen G, Halvorsen B, Attramadal H, Simonsen S, Frøland SS, Gullestad L, Christensen G, Damas JK, Aukrust P 2004 Elevated levels of activin A in heart failure: potential role in myocardial remodeling. *Circulation* 109:1379–1385.
- Florholmen G, Halvorsen B, Beraki K, Lyberg T, Sagen EL, Aukrust P, Christensen G, Yndestad A 2006 Activin A inhibits organization of sarcomeric proteins in cardiomyocytes during leukemia inhibitory factor. *J Mol Cell Cardiol* 41:689–697.
- Iezzi S, Di PM, Serra C, Caretti G, Simone C, Makani E, Minetti G, Zhao P, Hoffman EP, Puri PL, Sartorelli V 2004 Deacetylase inhibitors increase muscle cell size by promoting myoblast recruitment and fusion through induction of follistatin. *Dev Cell* 6:673–684.
- Sharma M, Kambadur R, Matthews KG, Somers WC, Devlin GP, Conaglen JV, Fowke PJ, Bass JJ 1999 Myostatin, a transforming growth factor- β superfamily member, is expressed in heart muscle and is upregulated in cardiomyocytes after infarct. *J Cell Physiol* 180:1–9.
- Morissette MR, Cook SA, Foo S, McKoy G, Ashida N, Novikov M, Scherzer-Croble M, Li L, Matsui T, Brooks G, Rosenzweig A 2006 Myostatin regulates cardiomyocyte growth through modulation of Akt signaling. *Circ Res* 99:13–24.
- McKoy G, Bicknell KA, Patel K, Brooks G 2007 Developmental expression of myostatin in cardiomyocytes and its effect on foetal and neonatal rat cardiomyocyte proliferation. *Cardiovasc Res* 74:304–312.
- Van Den Berg G, Somi S, Buffing AA, Moorman AF, Van Den Hoff MJ 2007 Patterns of expression of the follistatin and follistatin-like genes during chicken heart development: a potential role in valvulogenesis and late heart muscle cell formation. *Anat Rec (Hoboken)* 290:783–787.
- Adams D, Larman B, Oxbrough L 2007 Developmental expression of mouse Follistatin-like 1 (FSTL1): dynamic regulation during organogenesis of the kidney and lung. *Gene Expr Patterns* 7:491–500.
- Takehara-Kasamatsu Y, Tsuchida K, Nakatani M, Murakami T, Kurisaki A, Hashimoto O, Ohuchi H, Kurose H, Mori K, Kagami S, Noji S, Sugino H 2007 Characterization of follistatin-related gene as a negative regulatory factor for activin family members during mouse heart development. *J Med Invest* 54: 276–288.
- Felkin LE, Taegtmeyer AB, Barton PJ 2006 Real-time quantitative polymerase chain reaction in cardiac transplant research. *Methods Mol Biol* 333:303–330.
- Hall JL, Birks EJ, Grindle S, Cullen ME, Barton PJ, Rider JE, Lee S, Harwalkar S, Mariani A, Adhikari N, Charles NJ, Felkin LE, Polster S, Miller LW, Yacoub MH 2007 Molecular signature of recovery following combination left ventricular assist device (LVAD) support and pharmacological therapy. *Eur Heart J* 28:613–627.
- Wong K, Boheler KR, Petrou M, Yacoub MH 1997 Pharmacological modulation of pressure-overload cardiac hypertrophy: changes in ventricular function, extracellular matrix and gene expression. *Circulation* 96:2239–2246.
- Kogure K, Zhang YQ, Kanazaki M, Omata W, Mine T, Kojima I 1996 Intravenous administration of follistatin: delivery to the liver and effect on liver regeneration after partial hepatectomy. *Hepatology* 24:361–366.
- Bartholin L, Maguer-Satta V, Hayette S, Martel S, Gadoux M, Bertrand S, Corbo L, Lamadon C, Moreau AM, Magaud JP, Rimokh R 2001 FLRG, an activin-binding protein, is a new target of TGF β transcription activation through Smad proteins. *Oncogene* 20:5409–5419.
- McCroskey S, Thomas M, Platt L, Hennebury A, Nishimura T, McLeay L, Sharma M, Kambadur R 2005 Improved muscle healing through enhanced regeneration and reduced fibrosis in myostatin-null mice. *J Cell Sci* 118:3531–3541.
- Cohn RD, Liang HY, Shetty R, Abraham T, Wagner KR 2007 Myostatin does not regulate cardiac hypertrophy or fibrosis. *Neuromuscul Disord* 17:290–296.
- Hambrock HO, Kaufmann B, Muller S, Hanisch FG, Nose K, Paulsson M, Maurer P, Hartmann U 2004 Structural characterization of TSC-36/Flik analysis of two charge isoforms. *J Biol Chem* 279:11727–11735.
- Schellings MW, Pinto YM, Heymans S 2004 Extracellular proteins in the heart: possible role during stress and remodeling. *Cardiovasc Res* 64:24–31.
- Sage EH, Reed M, Funk SE, Truong T, Steadale M, Puolakkainen P, Maurice DH, Bassuk JA 2003 Cleavage of the extracellular matrix protein SPARC by matrix metalloproteinase 3 produces polypeptides that influence angiogenesis. *J Biol Chem* 278:37849–37857.
- Kawabata D, Tanaka M, Fujii T, Umebara H, Fujita Y, Yoshifuji H, Mimori T, Ozaki S 2004 Ameliorative effects of follistatin-related protein/TSC-36/FSTL1 on joint inflammation in a mouse model of arthritis. *Arthritis Rheum* 50:660–668.
- Miyamae T, Marinov AD, Sowders D, Wilson DC, Devlin J, Boudreau R, Robbins P, Hirsch R 2006 Follistatin-like protein-1 is a novel proinflammatory molecule. *J Immunol* 177:4758–4762.
- Ohshima Y, Ouchi N, Sato K, Pimentel RD, Izamiya Y, Schiekofer S, Walsh K 2007 Cardiac-protective action of follistatin-like-1, a secreted anti-apoptotic factor regulating Akt. *Circulation* 116:1541–1541 (Abstract).
- Shi Q, Bao S, Maxwell JA, Reese ED, Friedman HS, Bigner DD, Wang XF, Rich JN 2004 Secreted protein acidic, rich in cysteine (SPARC), mediates cellular survival of gliomas through Akt activation. *J Biol Chem* 279: 52201–52209.
- Mukherjee A, Sidis Y, Mahan A, Raher MJ, Xia Y, Rosen ED, Bloch KD, Thomas MK, Schneyer AL 2007 FSTL3 deletion reveals roles for TGF- β family ligands in glucose and fat homeostasis in adults. *Proc Natl Acad Sci USA* 104:1348–1353.
- Tortorello DV, Sidis Y, Holtzman DA, Holmes WE, Schneyer AL 2001 Human follistatin-related protein: a structural homologue of follistatin with nuclear localization. *Endocrinology* 142:3426–3434.

Endocrinology is published monthly by The Endocrine Society (<http://www.endo-society.org>), the foremost professional society serving the endocrine community.